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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Hendrik Louis BIJL et al.

Serial No.: 08/821,025

Filing Date: March 19, 1997

For: PROCESS FOR THE PREPARATION OF A GRANULAR MICROBIAL BIOMAS AND ISOLATION OF A COMPOUND THEREFROM

Examiner: Unassigned

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SUBMISSION OF CERTIFIED FOREIGN PRIORITY DOCUMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The filing papers claimed priority under 35 U.S.C. § 119 on the basis of European patent application no. 96200837.1, filed on 28 March 1996. Pursuant to 35 U.S.C. § 119, a certified copy of said European patent application is submitted herewith, thereby perfecting the priority claim.

☒ The issue fee has not become due for this application.

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The Assistant Commissioner is hereby authorized to charge any additional fees under 37 C.F.R. §§ 1.16 and 1.17 that may be required by this submission, or to credit any overpayment, to **Deposit Account No. 03-1952**.

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Respectfully submitted,

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Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

96200837.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Process for the isolation of valuable compounds from microbial biomass

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Process for the isolation of valuable compounds from microbial biomass

Field of the invention

The present invention relates to a process for the preparation of microbial biomass. Said microbial biomass is prepared in such a way that the isolation of a valuable product from the biomass is significantly improved.

Background of the invention

For a long time, microorganisms are known as a valuable source for a varying range of products. Several of these valuable microbial products are located inside or associated with the microbial cell. To recover such a product in a relatively pure form, it is necessary to separate said product from the microbial biomass. For instance, to isolate lipophilic compounds from microbial biomass, an extraction (leaching) step with an organic solvent or a supercritical fluid is performed.

The biomass material which is used for the extraction process usually is a wet cell cake which is pretreated to cause a significant disruption of the cell. Cell disruption can occur by physical treatment (e.g. drying, such as spray drying or lyophilization, and/or mechanical disintegration, such as crumbling or milling), by chemical (acid or alkaline) or by enzymatical treatment. Drying of the biomass is desirable to reduce the amount of solvents and, in case of extraction of lipophilic compounds, to prevent troublesome emulsions.

Most often, spray drying is applied to obtain biomass which is suitable for extraction. The drying by spray-driers requires the following conditions: to feed the dryer, the biomass should be a (concentrated) liquid or slurry with small particles (to enable the spraying by a disc or a nozzle). This means that for microbial biomass the

maximal dry matter concentration of the slurry is limited to approx. 20-30%, implicating that the subsequent drying step to reach a dry matter content of 90-95% will be relatively expensive.

The spray drying process enables to dry at short residence times, however, the product temperature in most cases is (relatively) high. This implicates there is a fairly large oxidation risk for oxidation-sensitive compounds. In case of biomass with high amounts of intracellular lipid material, the drying sometimes is troublesome due to sweating of the lipid from the biomass at higher temperatures. In this case extreme fouling of the dryer will occur.

Another drawback of spray drying is that this drying process puts a significant limitation to the type of extraction process which can be used to subsequently isolate a valuable compound. It is hardly posssible to apply percolation extraction on fine-powdered products such as spray dried biomass.

Mechanical disruption of the microbial biomass by milling also has the drawback of producing a large amount of fines.

In the British patent 1 466 853, a process to isolate oil from spray-dried yeast powder is described. The spray-dried yeast powder is wetted and heated by steam at 80°C in a cooker. The wet powder then was fed in a pelletising machine, converted to pellets, flaked and dried again to reduce the moisture content to 5-10% by weight. The resulted flakes then were filtered by a screen and the fines were recycled to the pelletiser. This material was suitable for percolation extraction. This method has the drawback that the dried powder has to be rehydrated, treated at high temperatures and redried. For thermo- and/or oxidation sensitive compounds, such a treatment has to be avoided as much as possible.

International patent application WO93/25644 discloses a method for the isolation of lipid with a high content of polyunsaturated fatty acids, from macro- and/or microalgae, whereby the macroalgae can be residues from alginate or carrageenan extraction. The algae should have particle size of less than 50 mm and a dry matter content of more than 50%. With algal material having a particle size larger and/or a dry matter content less than the indicated values, the material should pretreated by milling and/or drying. As indicated above, milling is disadvantageous for an efficient extraction process.

Summary of the invention

The present invention discloses a process for the isolation of a compound from microbial biomass, wherein the biomass is granulated to obtain biomass with a discrete particle structure and size and wherein said granulated biomass is subsequently dried to a dry matter content of at least 80%.

Preferably, the biomass is granulated using an extrusion process.

The process of the invention is especially suitable to isolate lipophilic compounds, such as polyunsaturated fatty acid-containing lipids or carotenoids, from microbial biomass.

The compounds isolated according to the process of the invention are suitable for human or animal food use and for use in cosmetics.

Brief description of the figures

Figure 1 shows the drying behaviour of different amounts of extruded biomass at different temperatures.

Figure 2 shows the extraction of oil from extruded biomass at different temperatures.

Figure 3 shows a diagram of a percolation extraction process.

Figure 4 shows the relation between amount of extracted oil and time of extraction.

Detailed description of the invention

The present invention shows that the pretreatment of microbial biomass cake to form granular particles having a discrete structure and size significantly improves the subsequent drying process. The resulting dried granulated biomass is very suitable for either immersion or percolation extraction. According to the process of the invention, the particle size can be specifically adjusted for an optimal drying and extraction result. Using biomass pretreated according to the invention, a valuable compound is advantageously extracted without the need to disrupt the cells prior to extraction.

Furthermore, the relatively mild conditions of the process of the invention are essential to ensure as less as possible degradation of a valuable compound which is

labile.

In the process of the invention, a microorganism of choice is first fermented to obtain a sufficient amount of biomass for subsequent extraction of the valuable compound. The fermentation conditions will depend on the organism in question. The fermentation conditions may be optimized for a high content of the valuable compound in the resulting biomass.

After ending of the fermentation process, the fermentation broth optionally, depending on the the type of compound to be isolated, may be pasteurized to kill the production organism and to inactivate undesirable enzyme activities. If desired, flocculation agents and/or other processing aids may be added to the broth to improve its filterability.

In a subsequent step, a solid-liquid separation step is performed to separate the biomass from the fermentation broth. The harvested biomass then usually has a dry matter content varying from about 20 to about 35%, depending on the type of microorganism.

However, for extrusion and subsequent drying the biomass typically should have a dry matter content which ranges from 30 to 70%.

If the water content of the harvested biomass is too high for extrusion and subsequent drying, the biomass is subjected to an additional dewatering step. Any dewatering method known to the skilled person to result in the desired dry matter content of 30 to 70% can be used. Preferably, a mechanical dewatering method is used.

The maximal dry matter content which can be reached by mechanical dewatering varies depending on the type of microorganism. For certain microorganisms, e.g. yeast, the dry matter content of the biomass after mechanical dewatering will not exceed a level of about 35-40%, while the same process executed on biomass of certain lipid-rich microorganisms, may result in a dry matter content of 45-55%.

A membrane filter press (plate and frame filter press with squeezing membranes) combines a solid-liquid separation with a mechanical dewatering step and is especially suitable to obtain the desired dry matter content.

Alternatively, the desired dry matter content of the microbial biomass can be increased by the addition of consistency-increasing agents before the extrusion step.

These consistency-increasing agents should be dry and should be chosen such that they do not negatively interfere with the extraction process and/or the properties of the isolated compound. For example, the consistency-increasing agents can be starch and/or plant fibres such as oats or wheat bran or cellulose, and the like.

After solid-liquid separation / mechanical dewatering, the biomass usually consists of large cakes, which cannot directly be used for extrusion. To reduce the biomass to a size which enables efficient feeding of the extruder, the dewatered biomass is crumbled, kneaded or mixed. Said crumbling or kneading can occur for instance by a short treatment in a high shear mixer. Optionally, the consistency-increasing agents may be added during the kneading process.

The crumbled or kneaded biomass is subsequently subjected to a granulation process to ensure the formation of material having a discrete particle structure and size.

In a preferred embodiment of the invention, the desired particle structure and size is obtained by an extrusion process. The particle characteristics with respect to structure and size are of great importance to obtain an efficient drying and extraction process. At the drying step, particles which are too small will give problems in generating dust and fines, whereas too large particles do not fluidize and give a bad drying performance. In extraction, a too small particle size does not enable the use of a percolation process, since the pressure drop over the biomass bed will be too high giving a high risk for the breaktrough of fines through the biomass bed. Too much fines will give problems in subsequent purification steps. A too large size impedes efficient penetration of solvent during extraction. Furthermore, the particle structure should be sufficiently compact to prevent disintegration during drying and extraction, but the particles should also have the proper porosity, to allow efficient penetration of solvent during extraction.

The extrusion conditions can be adjusted according to the knowledge of the skilled person to obtain biomass particles having the desired structure and size.

The extrusion conditions can be further adjusted to minimize cell disruption. Minimal cell disruption ensures optimal protection of labile, oxidation-sensitive compounds against oxidation-induced degradation.

Throughout the invention, a discrete particle structure and size is meant to encompass biomass particles which have a diameter of 0.3 to 10 mm, preferably, a

diameter of 1 to 3 mm, and which have a length approximately 2 to 6 times the diameter. Commonly, the particles will automatically obtain the desired length. Otherwise, the particles may be cut to the desired length.

The present invention also envisages other granulation methods which enable the formation of discrete particles. For instance, a multistage drying process comprising a combination of spray-drying and a fluidized bed also yields a granulate.

Optionally, antioxidants may be added prior to the granulation process.

In the next step, the extruded or otherwise granulated biomass is dried under conditions that the particles are kept intact. The discrete particle structure and size of the biomass after the granulation process specifically enables the efficient drying of said biomass. The drying can be performed using various dryers, e.g. a belt dryer, a vacuum or a vacuum belt dryer, a fluidized or a subfluidized bed dryer. The skilled person can additionally choose between a batch or a continuous process.

The use of a fluidized or subfluidized bed dryer is especially preferred in the process of the invention. Drying can occur under air, under nitrogen or under vacuum. With fluidized and subfluidized bed drying, the temperature in the bed can be adjusted to preset values. Said values can widely range, from about 35° to 120° C. If a labile compound needs to be isolated from the biomass, the temperature of the drying process can easily be adjusted to the lower ranges, to diminish the risk of oxidation or degradation.

Several advantages are conceivable which stress the importance of a drying step. First of all, drying of the biomass particles results in an intermediate material which can be stably stored for a prolonged time period, whereby a high dry matter content of the biomass prevents degradation of a valuable compound to be isolated from the biomass. In this way, the dried extrudate or granulate can be considered as a stable formulation of a compound present within or associated with the biomass.

For instance, the extrudate can function as a carrier for an enzyme, whereby the enzyme is immobilized within the extrudate by mixing an appropriate amount of a cross-linking agent, e.g. glutaraldehyde into the biomass before extrusion.

In addition, the dried granulated biomass prepared according to the invention can be advantageously used as such, for instance as a food or feed composition or additive.

In particular, the process of the invention results in biomass material with characteristics especially enabling a cost-effective and efficient extraction of valuable compounds. For instance, the process of the invention enables the efficient use of a percolation extraction process, whereby the advantages in the extraction process are due to the specific and discrete particle structure and size as well as the high dry matter content of the extrudate. A dry extrudate requires a reduced amount of solvent for the extraction of the valuable compound therefrom. In addition, the process of desolventizing toasting, i.e. the release of used solvent from the biomass, can be performed better and more efficient with biomass in the form of an extrudate.

The extrudate residue obtained after the process of desolventizing toasting can advantageously be used as a feed component.

A dry matter content of the extrudate exceeding 90-95% enables stable storage of the extrudate, whereas a dry matter content above 85% already gives a significant advantage in the subsequent extraction process.

The process of the invention can also be applied to obtain a mixture of valuable compounds from different microorganisms by preparing a granulate or extrudate from a mixture of two or more microorganisms. Said mixture of microorganisms can be obtained by mixing the fermentation broths of two or more different microorganisms directly after ending of the fermentation or by combining biomass from two or more microorganisms immediately prior to the granulation or extrusion process. It is also possible to mix two or more microbial extrudates prior to the extraction process.

The process of the invention is suitable to prepare an extrudate or granulate from any type of microorganism, whereby said microorganism can be in a filamentous form, like fungi or certain bacteria, or as single cells, like yeasts, algae and bacteria.

The valuable compound to be efficiently isolated from microbial biomass prepared according to the invention can be located intracellularly, associated with the cell membrane or cell wall, or produced extracellularly but insoluble in water.

The compound to be isolated from said biomass can further be either a hydrophilic or a hydrophobic c.q. lipophilic compound. Examples of such compounds are intracellular proteins or enzymes, lipids, various secondary metabolites like vitamins, macrolide or polyene antibiotics, flavours, carotenoids. Preferably, the compound to be isolated from microbial biomass is a lipophilic compound.

The product which is extracted from the biomass pretreated according to the method of the invention has a quality which is hardly subjected to any form of deterioration due to the mild conditions of the pretreatment process. Therefore, the method of the invention is very suitable for the preparation of microbial biomass from which heat- and/or oxidation-sensitive compounds need to be isolated.

The method of the invention is especially suitable to prepare microbial biomass for the isolation of compounds having a high degree of unsaturation, such as lipids containing polyunsaturated fatty acids. For instance, for the isolation of docosahexaenoic acid-containing lipid from algae or fungi, such as the dinoflagellate *Crypthecodinium* or the fungus *Thraustochytrium*, for the isolation of γ-linolenic acid-, dihomo-γ-linolenic- or arachidonic acid-containing lipid from fungi, such as *Mortierella*, *Pythium* or *Entomophthora*, or for the isolation of eicosapentaenoic acid-containing lipid from algae, such as *Porphyridium* or *Nitzschia*.

Specific additional examples of valuable compounds which are advantageously isolated according to the invention include, ß-carotene from fungal genera from the order Mucorales, e.g. *Phycomyces* or *Blakeslea*, astaxanthin from the yeast *Phaffia rhodozyma*, tetraacetylphytosphingosine from the yeast *Pichia ciferrii*, vitamin B12 from propionic acid bacteria.

The valuable compounds which are isolated according to the invention have a high quality and are suitable for human or animal nutrition. Especially, polyunsaturated fatty acid-containing lipids isolated according to the invention are suitable for nutritional purposes, in particular for the incorporation in infant formula.

Example 1

A comparison of solid/liquid separation performed with different methods

Decanter:

350 l of broth obtained from a typical fermentation of *Mortierella alpina* was decanted in the 'FLOTTWEG' decanter (type Z 23-3/441). The speed was set at about 4000 rpm. The differential speed range was varied during operation from 7.5 - 20 rpm.

The feed was set on 400 l/h. The biomass was not washed. In total 350 l broth

was decanted. The temperature of the feed was 8 °C and of the supernatant 15 °C. The dry matter content of the recovered biomass was about 25 %.

Decanter + vacuum drum filter:

20 kg of the biomass from the decanter experiment above with a dry matter content of 25 % was suspended in 500 l mainswater in which 10 kg NaCl was dissolved. The resulting slurry was filtered on a vacuum drum filter with belt discharge (clothtype: 865.912 K/5 polyprop) without further washing. The speed of the drum was set on 1 rpm and the pressure difference on a maximum of 600 mbar. In total 400 l was filtered within 15 minutes. The net filtering surface was about 0.3 m^2, which resulted in an average flow of 5000 l/m^2h (filtering surface).

The filtration rate was very well but the 'cake building' was rather bad. The dry matter content of the recovered filtered biomass was about 35 %.

Plate and frame filterpress:

500 l of broth was filtered in a plate and frame filterpress (cloth type: nycot 2794). The broth was filtered with a pressure difference of 0.3 bar. Within 35 minutes 500 l broth was filtered over a total filter area of 5 m^2, which resulted in an average flow of ± 175 l/m^2h. The filter cake was washed in 30 minutes with about 10 cakevolumes of mainswater which resulted in an average flow of 400 l/m^2h.

The cake was blown dry by air for 30 minutes, which resulted in a dry matter content of the recovered biomass of about 25 %.

Membrane filterpress:

500 l of broth was filtered in a membrane filterpress (cloth type: propex 46K2). The broth was filtered with a pressure difference of 0.2 bar. Within 21 minutes 500 l broth was filtered over a total filter area of 6.3 m^2 which resulted in an average flow of about 230 l/m^2h. The filter cake was washed in 30 minutes with 10 cake volumes of mainswater, which resulted in an average flow of 320 l/m^2h.

The advantage of a membrane filterpress above a plate and frame press is that the cake after filtration can be squeezed at high pressure, so the dry matter content of the cake will increase. The cake was squeezed at 5.5 bar during 30 minutes which

resulted in a dry matter content of the recovered biomass of about 45 %.

In another experiment, the filter cake was washed in 18 minutes with 3 cake volumes of a 1 % NaCl solution, which resulted in an average flow of 390 l/m^2h. The cake was squeezed at 6 bar during 30 minutes, which resulted in a dry matter content of the recovered filtercake of about 55 %.

Both squeezing as well as washing of the cake with a 1 % salt solution has a significant effect on the dry matter content of the filtercake.

Example 2

Extrusion of biomass with different dry matter contents

Extrusion was performed with biomass with different dry matter contents, which were obtained by the methods presented in Example 1 (see Table 1). Extrusion was performed using a single screw extruder with a profiled barrel and a universal screw.

The dieplates applied in extrusion had a different number of holes and the diameters of the holes were in the range of 1 -3 mm. The diameter of the particles obtained after extrusion was about 2 mm.

The performance and extrudate quality is depending on the percentage dry matter of the biomass used for extrusion.

Table 1. Results of extrusion experiments with biomass with different dry matter contents.

% Dry matter	Performance of extrusion	Quality of extrudate
25	bad	very sticky material
35	good	sticky material
45	very good	non sticky extrudate
55	very good	loose extrudate

Example 3

Drying of conventional and extruded biomass of *Mortierella alpina*

Vacuum drying:

Conventionally recovered biomass was dried in a vacuum tray dryer during ± 50 hours at 40 °C. The drying was very slow because of lumps. The dry matter content of in this way dried biomass was about 92.5 %.

About 20 g of extrudate (Example 2, $\varnothing_{particle}$ of 2 mm) with a dry matter content of 55 % was dried on labscale in a rotavapor. The temperature of the waterbath was 68 °C and the applied pressure 40 mbara. The performance of the drying was reasonable, except that the dried biomass sticks to the wall and sweats oil. The dry matter content after drying was 92.3 %.

Fluidized bed drying:

Drying was performed with biomass at different temperatures. In case that no pretreatment of the biomass has occurred, big lumps of biomass do not become completely dry. In this case the dried biomass was very inhomogeneous considering the particle size.

If the biomass was pretreated before drying by means of extrusion, the performance of drying substantially improved. In this case the particle size of the dried biomass is more uniform.

The conclusion of these results is that fluidized bed drying can be performed with different forms of isolated biomass, but that drying will be improved using an extrudate.

In another experiment, drying of different quantities (15 and 30 kg) extrudate was performed in a fluidized bed dryer with air (8000 Nm^3/m^2h). During the drying experiment samples were taken and the dry matter content was estimated. In Fig. 1 the relationship between temperature and dry matter content of the different quantities is shown. The setpoint of the bed temperature was set on 80 °C. The diameter of the extruded biomass was 1.3 mm. The dry matter content of the extruded biomass after drying is about 96%.

Example 4

Extraction of lipid from dried extrudate of *Mortierella alpina*

Stirred extraction of dried extrudate at different temperatures:

Samples of 100 g of dried extrudate with respectively 93.4 and 97.8 % dry matter were extracted during 3 hours with 500 ml hexane or 500 ml propanol-2, at temperatures of 20°, 35° and 50° C for hexane and 20°, 40° and 70° C for propanol-2. The slurry was stirred by means of a two blade stirrer in a 'four-necked' roundbottom flask and heated by means of a heating mantle. Eventually evaporated hexane or propanol-2 was recycled by means of a reflux cooler.

During the extraction, every 30 minutes a 15 ml sample of the supernatant was taken from the flask after the stirrer was stopped and the particles were settled.

1 ml of the samples was pipetted in preweighed 2 ml eppendorf tubes. After overnight drying under vacuum at 40 °C the eppendorf tubes were weighed and total oil was calculated.

The results of the experiments are shown in Fig. 2.

Conclusion hexane extraction:

- the temperature had no effect on the total amount of lipid that can be extracted, i.e. a relatively low extraction temperature gives a good yield of lipid,

- the temperature had only a small effect on the time in which the total amount of lipid can be extracted,
- the total amount of lipid is extracted within 30 minutes from the biomass, with 5 volumes of hexane at a temperature above 20 °C.

Conclusion propanol-2 extraction:

- the temperature had a significant effect on total amount of lipid that can be extracted,
- the temperature had a significant effect on the time in which the total amount of lipid can be extracted,
- the total amount of lipid is extracted within 2 hours from the biomass with 5 volumes of propanol-2 at 73 °C.

The composition of the oil depends on the solvent used in extraction (see Table 2). The more polar the extraction solvent the more phospholipids are extracted.

Table 2 Extraction of dried *Mortierella* biomass at room temperature using two different solvents.

Substance	hexane oil	propanol-2 oil
Triglycerides	93 %	85 %
diglycerides	2 %	2 %
monoglycerides	2 %	2 %
sterols	3 %	3 %
phospholipids	2 %	6.5 %

On larger scale problems were observed with the filtration of the micella, due to disintegration of the extrudate into small particles due to the high stirrer speed during the extraction process.

These problems can be avoided by the application of percolation extraction instead of stirred extraction.

Percolation extraction of dried extrudate with hexane:

Several percolation extractions were performed on pilotscale (see Fig. 3 for a diagram of the applied process).

About 40 - 45 kg of dried extruded biomass was extracted with hexane (initial hexane/biomass ratio of 4.4 l/kg) at 20 °C. The flow of the gearpump was set on 1.5 m^3/h. There was a small N_2 purge on holdup vessel of about 0.1 bar.

The extraction was performed during 4 hours (temperature increase during extraction from 18 to 20.5 °C). Each 30 minutes samples were taken from the micella. Of each sample, 100 ml was evaporated at labscale in a rotavapor ($T_{waterbath}$ was 64 °C) during 20 minutes under vacuum (about 50 mbar). The amount of oil was estimated. The results are presented in Fig. 4.

It can be noticed that after 2 hours an 'equilibrium' was reached. Afterwards, the extracted biomass was washed with about 0.6 bedvolumes of hexane. During the extraction the bed height did not change.

The micella were polish filtered prior to evaporation. During the extraction we noticed that the micella became more and more clear, due to depth-filtration over the bed of particles.

Example 5

Recovery of a crude β-carotene oil from *Blakeslea trispora*

10 l of a typical fermentation broth of the fungus *Blakeslea trispora* was harvested using laboratory filtration equipment. To improve the filterability of the broth $CaCl_2$ was added (end concentration of 0.5 g/l). In this way recovered biomass was mechanically dewatered at labscale up to a 45% dry matter content using a typical fruit press (citrus press). The cake recovered in this way was extruded by means of a single screw lab extruder using a universal screw and a profiled barrel. The diameter of the four holes in the dieplate was 2 mm. The resulting extrudate was dried in a labscale fluid bed dryer (T_{air} = 35 °C, drying time of 25 minutes, airflow of 45 Nm^3/h). The dry matter content of the biomass dried in this way was about 85 %.

A sample of about 100 g of dried extrudate was extracted using percolation extraction with ethyl acetate (initial volume/biomass ratio of 4 l/kg). After 2 hours of

extraction at 50 °C the micella was harvested by means of vacuum filtration. The micella recovered in this way was evaporated at 50° C ($T_{waterbath}$). In this way a crude β-carotene containing oil was recovered.

Example 6

Extrusion of biomass from the yeast *Pichia*

Biomass from 2 l of a fermentation broth of the yeast *Pichia ciferrii* was harvested using pressure filtration. The filtercake was washed with a salt solution containing 1 % NaCl. The biomass recovered in this way was mechanically dewatered at labscale using a typical fruit press. During one minute the cake was dewatered at 200 kg/cm^2.

The dewatered cake was extruded by means of a single screw laboratory extruder using a universal screw and a profiled barrel. The diameter of the hole(s) in the dieplate was varied from 1 - 2.5 mm.

The resulting extrudate was dried in a labscale fluid bed dryer. The temperature of the applied air was 60 °C. After 60 minutes drying at this temperature, the dry matter content of the biomass was about 95 %. The biomass processed in this way is very suitable for the hexane extraction of tetraacetyl-phytosphingosine.

Example 7

Recovery of astaxanthin from the yeast *Phaffia*

Biomass from 5 l of a fermentation broth of the yeast *Phaffia rhodozyma* was harvested using vacuum filtration. The filter cake was washed with a salt solution containing 1 % NaCl. The filter cake recovered in this way was extruded by means of a single screw lab extruder using a universal screw and a profiled barrel. The diameter of the hole(s) in the dieplate was varied from 2 - 3 mm.

The resulting extrudate was dried in a labscale fluid bed dryer. The temperature of the applied air was 40 °C. After 90 minutes drying at this temperature, the dry matter content of the biomass was about 96 %. The biomass processed in this way is suitable for solvent extraction of astaxanthin.

A sample of about 200 g of dried extrudate was extracted using percolation extraction with hexane (initial volume/biomass ratio of 4 l/kg). After 2 hours of extraction at ambient temperature, the micella was harvested by means of centrifugation.

The micella recovered in this way was evaporated at 60 °C ($T_{waterbath}$). In this way crude astaxanthin was recovered.

Example 8

Recovery of DHA oil from *Crypthecodinium*

Biomass from 10 l of a typical fermentation broth of the alga *Crypthecodinium cohnii* was harvested using filtration equipment. The in this way recovered biomass was mechanically dewatered at labscale using a typical fruit press. During 1 minute the cake was pressed at 300 kg/cm^2. The cake recovered in this way was extruded by means of a single screw lab extruder using a universal screw and a profiled barrel. The diameter of the hole(s) in the dieplate was varied from 1 - 4 mm. The resulting extrudate was dried under vacuum overnight at 40 °C.

The dry matter content of the biomass dried in this way was about 94 %.

A sample of about 100 g of dried extrudate was extracted using percolation extraction with hexane (initial volume/biomass ratio of 5 l/kg). After 2 hours of extraction at 50 °C the micella was harvested by means of centrifugation in a Beckmann centrifuge (10 minutes at 4000 rpm).

The micella recovered in this way was evaporated at 50°C ($T_{waterbath}$). In this way a crude DHA containing oil was recovered.

Example 9

Recovery of Vitamin B12 from *Propionibacterium sp.*

Biomass from 3 l of a flask fermentation of a *Propionibacterium sp.* was harvested using pressure filtration at 1 bar. To improve the filterability of the broth Al_2SO_4 was added (end concentration of 1 g/l). In this way recovered biomass was mechanical dewatered at labscale using a typical fruit press. During 1 minute the cake was pressed

at 300 kg/cm^2. The cake recovered in this way was mixed with 1 - 2 % of starch or wheat bran and extruded by means of a single screw lab extruder using a universal screw and a profiled barrel. The diameter of the hole(s) in the dieplate was varied from 1 - 4 mm. The resulting extrudate was dried under vacuum overnight at 40 °C. The dry matter content of the biomass dried in this way was about 94 %.

A sample of about 50 g of dried extrudate was extracted using percolation extraction with water of pH 5 (initial volume / biomass ratio of 5 l/kg). After 2 hours of extraction at 50 °C the micella was harvested by means of centrifugation in a Beckmann centrifuge (10 minutes at 4000 rpm).

Vitamin B12 can be recovered from the extract using ion-exchange chromatography.

Claims

1. A process for the isolation of a compound from microbial biomass, comprising the steps of:

- obtaining biomass with a dry matter content of 30 to 70%,
- granulating said biomass with a dry matter content of 30 to 70% to obtain biomass material having a discrete particle structure and size,
- drying said granulated biomass to a dry matter content of at least 80%, and
- extracting said compound from said granulated and dried biomass.

2. A process for the isolation of a compound from microbial biomass, comprising the steps of:

- obtaining biomass with a dry matter content of 30 to 70%,
- crumbling or kneading said biomass with a dry matter content of 30 to 70%,
- extruding the kneaded biomass to obtain biomass having a discrete particle structure and size,
- drying said extruded biomass to a dry matter content of at least 80%, and
- extracting said compound from said extruded and dried biomass.

3. A process according to claim 1 or 2 wherein the biomass with a dry matter content of 30 to 70% is obtained by solid/liquid separation.

4. A process according to claim 3 wherein the solid/liquid separation is combined with mechanical dewatering.

5. A process according to claim 3 wherein the biomass with a dry matter content of 30 to 70% is obtained by the addition of a solid material to the biomass.

6. A process according to any one of the claims 1 to 5 wherein the drying of the biomass to a dry matter content of at least 80% is performed by fluidized bed or subfluidized bed drying.

7. A process according to any one of the claims 1 to 6 wherein the biomass originates from a fungus.

8. A process according to claim 7 wherein the fungus belongs to the order Mucorales.

9. A process according to claim 8 wherein the fungus belongs to the genus *Mortierella*, preferably is *Mortierella alpina*.

10. A process according to any one of the claims 1 to 6 wherein the biomass originates from an alga.

11. A process according to claim 10 wherein the alga is a dinoflagellate, preferably belongs to the genus *Crypthecodinium*, most preferably is *Crypthecodinium cohnii*.

12. A process according to any one of the claims 1 to 11 wherein the compound is a polyunsaturated fatty acid-containing lipid.

13. A process according to claim 12, wherein the polyunsaturated fatty acid is selected from the group comprising C18, C20 and C22 ω-3 and C18, C20 and C22 ω-6 polyunsaturated fatty acids, preferably from the group of C20 and C22 ω-3 and C20 and C22 ω-6 polyunsaturated fatty acids, more preferably from the group of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid.

14. A process according to claim 8 wherein the fungus belongs to the genus *Phycomyces* or *Blakeslea*.

15. A process according to any one of the claims 1 to 11 and 14 wherein the compound is a carotenoid.

16. A microbial extrudate which is obtained from *Mortierella*.

17. A process for the preparation of a food composition or a nutritional supplement comprising the use of a compound isolated according to the process of any one of the claims 1-15.

18. A process according to claim 17 wherein the food composition is an infant formula.

Process for the isolation of valuable compounds from microbial biomass

Abstract

The present invention describes a process for the isolation of a valuable compound from microbial biomass, wherein the microbial biomass has undergone a pretreatment to obtain a granulated biomass with a dry matter content of at least 80%. The granulation of the biomass to obtain particles having a distinct structure and a size significantly improves subsequent drying of the biomass as well as extraction of said valuable compound.

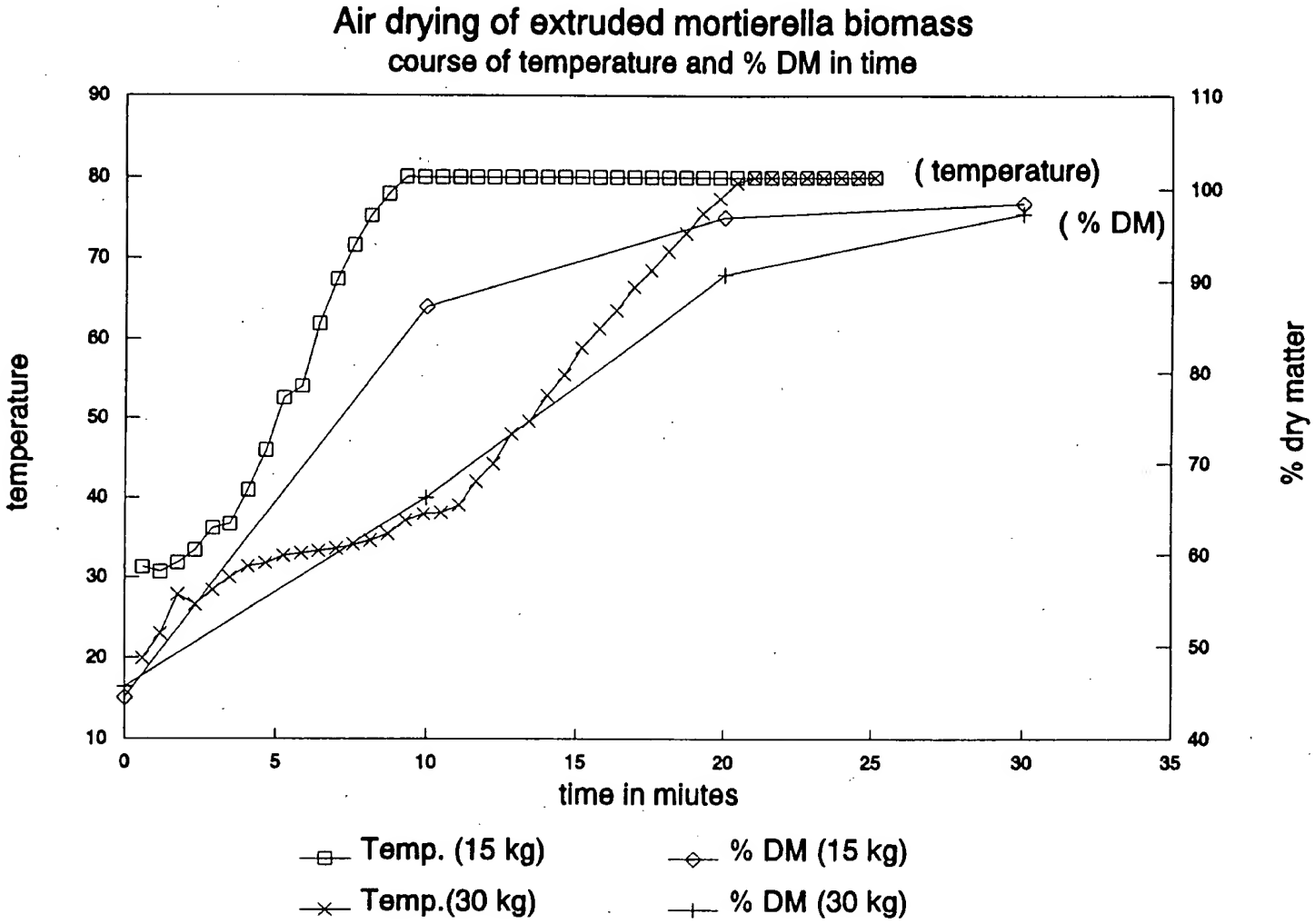

Air drying of extruded mortierella biomass
course of temperature and % DM in time
temperature
% dry matter
time in miutes
(temperature)
(% DM)
Temp. (15 kg)
Temp.(30 kg)
% DM (15 kg)
% DM (30 kg)

Fig. 1

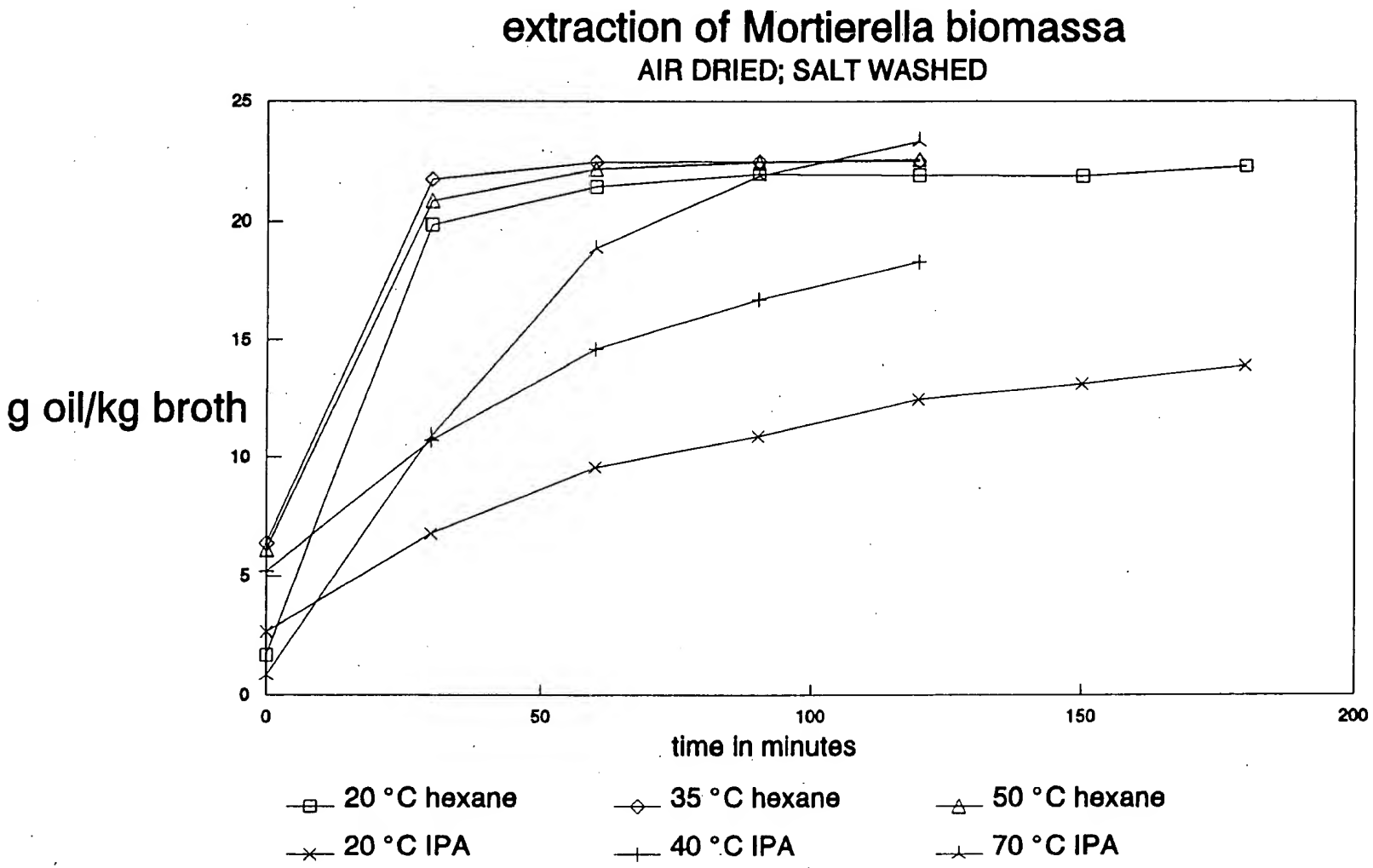
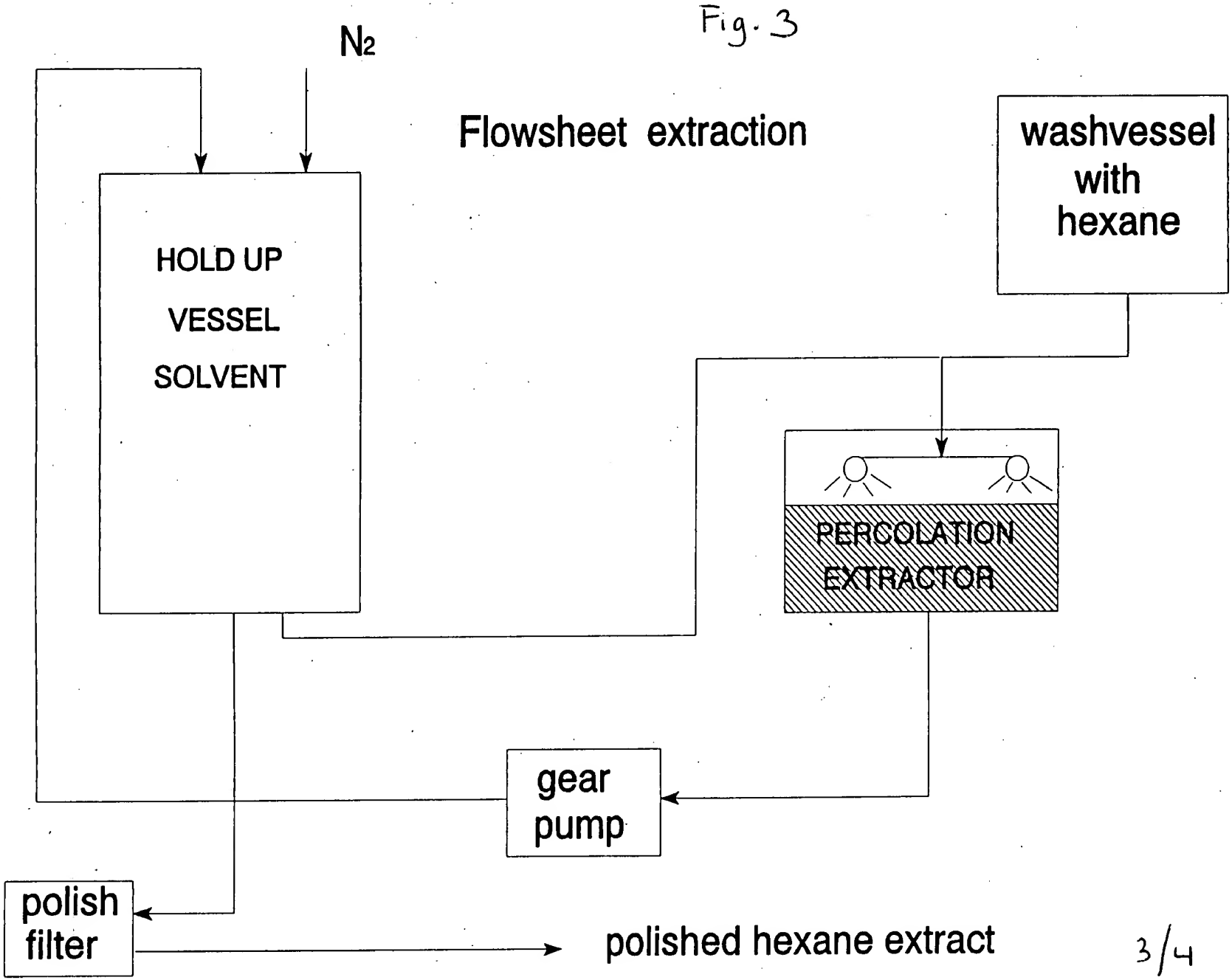

extraction of Mortierella biomassa
AIR DRIED; SALT WASHED
g oil/kg broth
25
20
15
10
5
0
0
50
100
150
200
time in minutes
20 °C hexane
35 °C hexane
50 °C hexane
20 °C IPA
40 °C IPA
70 °C IPA

Fig. 2

Fig. 3


N2
Flowsheet extraction
washvessel with hexane
HOLD UP VESSEL SOLVENT
PERCOLATION EXTRACTOR
gear pump
polish filter
polished hexane extract

Extraction of dried Mortierella biomass

Percolation extraction with hexane

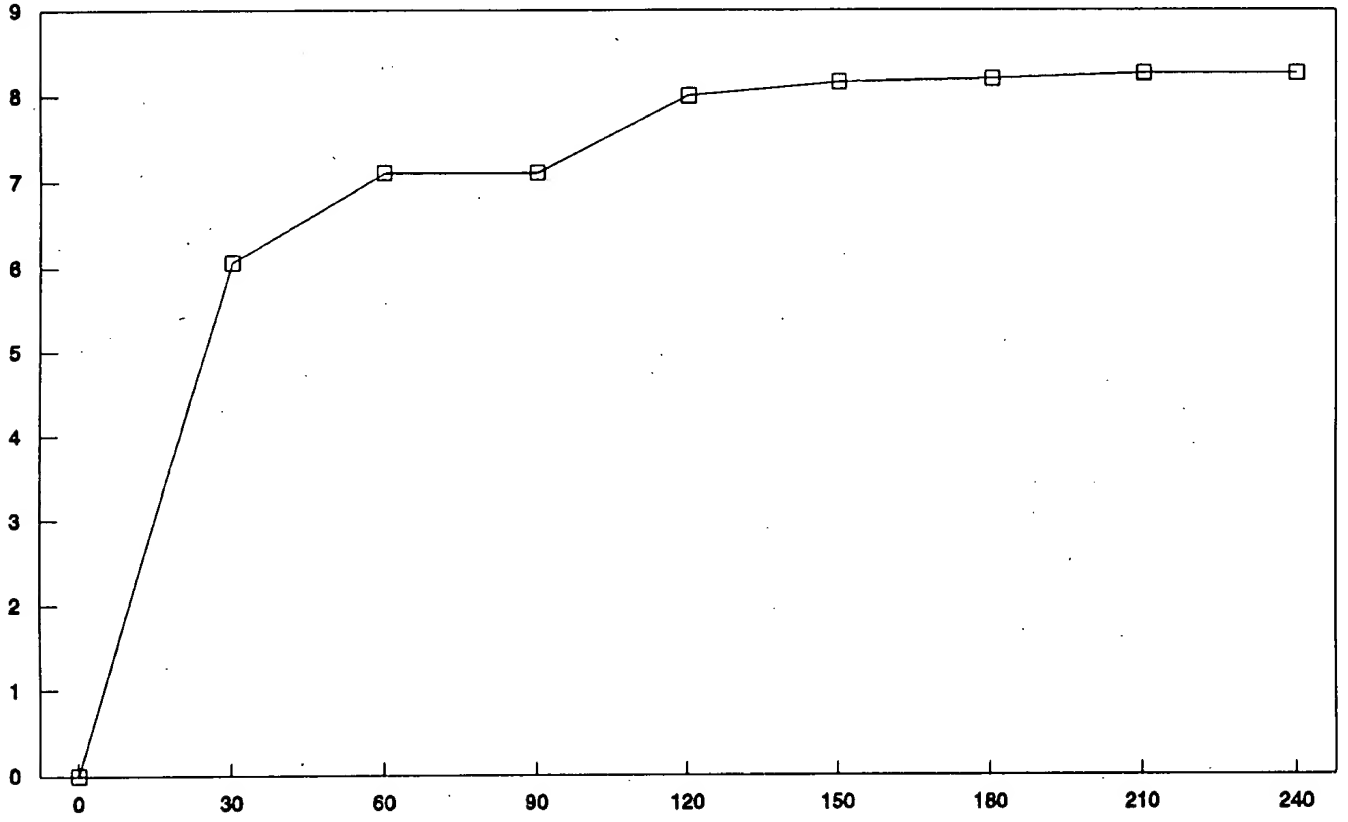


Fig. 4